612.744.2:547.781.5

APPEARANCE OF HISTAMINE IN THE VENOUS BLOOD DURING MUSCULAR CONTRACTION.

By G. V. ANREP and G. S. BARSOUM.

(*From the Physiological Laboratory, University of Cairo, Egypt.*)

(*Received August* 12, 1935.)

The suggestion that the hyperæmia which follows muscular contraction is due to the local production of some potent vaso-dilator substances was first made by Gaskell over fifty years ago [1877]. At that time no other metabolites were known besides those of an acid nature like lactic and carbonic acids, and the fact that acids produce in perfusion experiments a vaso-dilatation was taken as a sufficient proof in favour of the theory that the hyperæmia in active muscles is also caused by acids. This view assumed a further significance on account of the important role which lactic acid was shown to play in the intimate processes involved in muscular contraction. In the opinion of Krogh [1922] the concentration of these substances in the blood and the shift in the H-ion concentration produced by them is, under normal conditions, rarely large enough to affect the tone of the blood vessels. Krogh supplements the theory of acid metabolites by the suggestion that oxygen lack is the governing factor regulating the capillaries in the muscles. It is well known, however, that the blood flow through a muscle may increase during its rhythmical contractions so much that the outflowing blood loses to a considerable extent its venous character. The arterio-venous difference of the carbon dioxide concentration becomes reduced in spite of an increase in its production, and the blood emerging from the muscle during the hyperæmia is frequently better oxygenated than the resting blood. Lactic acid appears in the blood when the muscle runs into oxygen debt, but under normal conditions the working muscle gets a luxury supply of well-oxygenated blood.

The final proof that acid metabolites or oxygen lack are directly responsible for the hyperæmia has never been provided. The tacit acceptance of this view rests mainly on the knowledge that these factors are

connected with the process of muscular contraction, and that when they are made to act upon blood vessels they cause a vaso-dilatation. The inadequacy of this theory in its pure form is well shown in recent experiments of Keller, Loeser and Rein [1930], who measured the blood flow in the femoral artery by means of the thermostromuhr. Rhythmical contractions of only one of the leg muscles usually more than doubled the blood flow in the femoral artery. Injection of blood containing 0·05 p.c. of lactic acid directly into the femoral artery caused a much smaller increase in the blood flow. Injection of 0·15 p.c. did not increase the effect and a concentration of 0·5 p.c. had no vaso-dilator action. Rein tries to explain this result by suggesting that there must be a difference in the action of the acids when they are produced within the muscle and act mainly on the capillaries and when they are injected and allowed to act on the arteries as well.

A considerable advance in the study of this problem was made by Anrep and von Saalfeld [1935], who studied the vaso-dilator properties of the venous blood emerging from a muscle, not by means of a comparison with the action of some already known vaso-dilator substances but by measuring the vaso-dilator action of the venous blood itself. These experiments provided the first evidence showing that the blood emerging from a contracting muscle possesses strong vaso-dilator properties. With the help of their reperfusion method these workers have shown that the venous blood collected from a resting muscle produces, on reperfusion, no vaso-dilatation, and that even after the resting blood is reperfused through the muscle three times in succession the vaso-dilatation caused by it is small. On the other hand, the venous blood which is collected during periods of rhythmical contraction of the muscle produces, on reperfusion, a vaso-dilatation which in some instances may be as large as that accompanying the activity of the muscle. The chemical substances responsible for this action were shown to be stable in blood, and they were not destroyed by oxygenation of the blood.

Within recent years the whole subject of vaso-dilatation has undergone a complete revision in view of the discovery of a number of specific vaso-dilator substances which could not be grouped together with the usual acid metabolites. Adenosine, acetylcholine, histamine, sympathin I, and finally the vaso-dilator action of adrenaline—all these have now to be considered. Rigler [1932] suggested that an adenylic compound may play a role in the hyperæmia following muscular contraction. No direct proofs were, however, provided in support of this view. In order to test Rigler's suggestion one of us [G. V. A.] made in conjunction

with Prof. J. H. Gaddum some preliminary experiments, using the technique of Anrep and Saalfeld. The experiments gave rather inconclusive results, and they had to be interrupted on account of the temporary absence of one of the co-workers. Meanwhile Barsoum and Gaddum [1935 *a*] made a great advance in devising a physiological method for determination of histamine in small quantities of blood. In applying their method to the study of the hyperæmia which follows a temporary cessation of the blood flow through a limb they found that the venous blood contains, during the period of the hyperæmia, an increased amount of histamine. The periods of occlusion of the artery varied in their experiments from 5 to 60 min. The increase in the concentration of histamine was not always proportional to the duration of the occlusion, and the hyperæmia was not always proportional to the concentration of histamine. These experiments were of a preliminary nature [1935 *b*], and the results are probably in part vitiated by the insufficient precautions taken to abolish the collateral blood supply to the limb. The blood was collected from the whole limb and not from the muscle tissue alone. Nevertheless, the work of these observers is of an especial significance, since it brings the study of the problem in line with the researches of Sir Thomas Lewis [1927], who, barring the actual isolation of the histamine, advanced overwhelming evidence in favour of the important part which it plays in various vascular reactions of the skin.

In view of Prof. Gaddum's new appointment the partnership with him had to be broken, and by mutual consent he was replaced by his own partner in his work on the reactive hyperæmia. The technique employed in the experiments described in the present communication was precisely the same as that used by Anrep and Saalfeld; the method for the quantitative estimation of histamine in the blood was that of Barsoum and Gaddum. The blood samples were collected directly from the muscle vein in graduated cylinders containing a solution of trichloroacetic acid. The histamine was determined in samples of blood not less than 10 c.c. each. Dogs were used in all experiments. The animals were lightly morphinized before injection of ch'oralose which was used as an anæsthetic. Special care was taken during the preparation of the blood vessels of the muscle to abolish all the collateral communications between it and the rest of the circulation. A mixture of chlorazol fast pink and heparin was used as an anticoagulant.[1]

[1] The presence of chloralose, chlorazol fast pink and of heparin had no influence on the accuracy of the estimation of histamine. This was ascertained by experiments specially made for this purpose.

The normal arterio-venous histamine difference of muscle blood. Under normal conditions of the circulation through the muscle there is no appreciable difference in the concentration of histamine in the arterial and the venous blood. If, however, the blood flow through the muscle is diminished the venous histamine concentration rises above that of the arterial blood. The following experiments made on the gastrocnemius may serve as an illustrative example. The muscle was perfused by the animal's own blood-pressure from the femoral artery. During the periods of observation the arterial blood of the animal was collected in a 60 c.c. Dewar flask and then perfused through the muscle at any desired constant pressure. The flask was periodically refilled without interfering with the blood flow through the muscle except that it was momentarily reconnected to the femoral artery. The refilling of the reservoir never took more than 5 sec. After four or five perfusions of the muscle at the selected pressure the venous blood was collected and analysed. Samples of arterial blood were collected before and after the collection of the venous samples.

Blood-pressure in mm. Hg	Blood flow in c.c. per min.	Histamine in γ per c.c.
Exp. 1. Weight of the gastrocnemius, 34 g.		
Arterial sample	—	0·035
150	7·5	0·035
100	5·7	0·045
50	4·3	0·063
Arterial sample	—	0·035
Exp. 2. Weight of the gastrocnemius, 42 g.		
Arterial sample	—	0·022
45	3·1	0·045
85	6·5	0·030
160	11·7	0·022
Arterial sample	—	0·022

The above experiments were made with the muscle nerve intact, but the same results are obtained also after section of the nerve. In fact when, on account of an inadequate blood supply to the muscle, there is a higher concentration of histamine in the venous blood than in the arterial blood, this difference diminishes or even disappears due to the increase in the blood flow which takes place after the section of the nerve. For example, in one experiment the arterial histamine was 0·013 γ per c.c. and the venous was 0·025 γ. After the section of the nerve the blood flow increased by 25 p.c. while the histamine difference diminished by 60 p.c., the venous concentration decreasing to 0·020 γ. All the above figures are the result of triplicate determinations, none of which differed from the other more than 10 p.c. These experiments show that there is no strict inverse relationship between the blood flow and the arterio-venous

histamine difference. At a certain blood flow the excess histamine on the venous side disappears. No experiments were made yet in order to determine the conditions under which the muscle may take up histamine from the arterial blood.

The effect of rhythmical contractions of the muscle. In all the experiments (on 17 dogs), without a single exception, the venous blood collected during rhythmical contractions of the muscle contained an appreciably greater amount of histamine than the resting blood. Anrep and Saalfeld found that the vaso-dilator properties of the venous blood which is collected during the hyperæmia are quite definite, but that they become greatly increased if during the period of contractions the hyperæmia is not allowed to develop to its full extent. A small compression of the artery during the period of the hyperæmia leads to a considerable increase in the concentration of the vaso-dilator principles in the active venous blood. This observation was amply confirmed by the present experiments as can be seen from the following typical example.

Exp. 3. Weight of the gastrocnemius 30 g.

Description of the blood samples	Blood flow in c.c. per min.	Histamine in blood in γ per c.c.
Arterial blood	—	0·033
Resting venous blood	4·8	0·033
Rhythmical stimulation of the muscle nerve, blood flow not controlled	31·0	0·085
Immediately after	30·0	0·080
Resting venous blood	4·8	0·033
Stimulation as above but the blood flow is controlled	6·0	0·300
Immediately after	6·7	0·125
Arterial blood	—	0·025
Resting venous blood	3·6	0·030

The interesting part about this experiment is that the total amount of histamine which appears in the venous blood during the controlled and the non-controlled period of collection is exactly the same. The length and the strength of the stimulation of the nerve were the same in both cases. The arterio-venous histamine difference during the collection of the blood with a free circulation was 0·052 γ per c.c., which at the blood flow of 31 c.c. per minute gives an excess histamine of 1·61 γ per minute. During the controlled period the histamine difference was 0·267 γ per c.c., which at the blood flow of 6·0 c.c. per minute gives an excess of 1·60 γ per minute.

Other experiments gave results of the same kind except that they varied in the extent of the increase in the histamine concentration in the venous blood. The relative increase was the greater the more conspicuous the hyperæmia. For example, in one experiment in which the nerve had

been cut before the stimulation, the blood flow through the muscle was exceptionally large, 15 c.c. per minute. During the stimulation the free blood flow increased to a maximum of 33·5 c.c. per minute and the histamine concentration of the venous blood rose from 0·022 to 0·065 γ per c.c. In a few minutes the blood flow and the histamine of the venous blood returned to their resting values. In another experiment in which the blood flow was also large—13·8 c.c. per minute—it increased during the period of contractions to 52·4 c.c. per minute, while the histamine concentration increased from the already high level of 0·055 to 0·25 γ per c.c. The relatively larger increase in the blood flow was accompanied by a more conspicuous increase in the histamine concentration. The weight of the two muscles was the same within a few grams, and the stimulus applied was identical in both experiments.

The relation between the duration and strength of the contraction and the amount of histamine liberated by the muscle. Under normal conditions there is a definite dependence between the total amount of histamine set free as the result of the contraction of the muscle and the duration of this contraction. This can best be followed from the two experiments given below. One of them was made with a controlled blood flow, while in the other the blood flow was free. The only modification which was introduced in these experiments consisted in the collection of the entire active venous blood in one sample. The collection started 5 sec. before the beginning of the stimulation. A tetanic stimulus was then applied for a definite length of time, and the collection of the blood was continued until the blood flow returned to its resting value. An estimation of the histamine in this sample gives therefore the total excess of it on the venous side. It does not of course give any indication as regards the moment at which the maximal amount of the histamine is set free or produced by the muscle. In the experiment in which the blood flow was controlled and in which one could not be sure of the moment when the hyperæmia had come to an end, the blood was collected for some time in excess of the usual duration of the hyperæmia.

The excess histamine in the venous samples was calculated by subtracting the histamine per c.c. of the resting venous blood from that found in the active venous blood and then multiplying the difference by the number of c.c. of blood in the sample. The last column of the table shows the amount of histamine produced by 1 g. of muscle per minute of contraction. This figure is calculated on the basis of the excess histamine found in the samples of the active venous blood. For example, in the case of the 10-sec. tetanus in Exp. 5 the excess histamine was 3·48 γ.

Exp. 4. Weight of gastrocnemius, 25 g. Blood flow controlled. Weak stimulus evoking submaximal tetanus applied to muscle nerve. Stimulation began at the fifth second of collection of blood sample. Resting blood flow, 4·6 c.c. per min.; maximal blood flow during hyperæmia, 8·5 c.c. per min.

Description of the samples	c.c. of blood in sample	Histamine γ per c.c. of sample	Excess histamine in sample γ	Histamine per g. muscle per min. of contraction γ
Venous resting	11·0	0·018	—	—
Arterial	11·0	0·018	—	—
Tetanus for 5 sec.	13·0	0·040	0·286	0·137
„ 10 „	12·0	0·075	0·684	0·154
„ 15 „	10·5	0·125	1·124	0·180
Venous resting	12·0	0·018	—	—
Arterial	13·0	0·018	—	—

Exp. 5. Weight of gastrocnemius, 42 g. Blood flow not controlled. Stimulus evoking maximal tetanus applied to muscle nerve. The last stimulus was applied directly to the muscle: it evoked a maximal tetanus, but parts of the muscle remained relaxed.

Description of the samples	c.c. of blood in sample	Histamine γ per c.c. of sample	Excess histamine in sample γ	Histamine per g. muscle per min. of contraction γ
Arterial	12·0	0·025	—	—
Venous resting	10·0	0·025	—	—
Tetanus for 2 sec.	14·5	0·060	0·51	0·364
„ 5 „	22·5	0·100	1·68	0·480
„ 10 „	24·0	0·170	3·48	0·497
Arterial	12·0	0·025	—	—
Venous resting	10·5	0·025	—	—
Tetanus for 5 sec.	17·0	0·125	1·70	0·485
Direct stimulation of the muscle for 15 sec.	24·0	0·190	3·96	0·378

On multiplying this figure by 6 and dividing by 42, being the weight of the muscle, we arrive at the result given in the last column of the table. The figures given in the second column do not represent the maximal hyperæmia but the volume of the sample taken.

Several important deductions can be made from these experiments. It can be seen, for instance, especially from Exp. 4, that with prolongation of the tetanus the amount of histamine set free becomes progressively larger for every second of contraction. A tetanus lasting for 5 sec. was accompanied by an appearance of an excess histamine of 0·286 γ. Tetani lasting 10 and 15 sec. led to liberations of histamine which were respectively more than double and treble that figure. This relation is well shown in the last column of the table. In the second experiment this effect is more conspicuous at the shorter contractions of the muscle. It almost disappears when the tetanus lasts for more than 5 sec. Similar results were obtained in all the other experiments except that in some

the effect was more obvious than in others. Whether this difference depends on the condition of the muscle, on the initial blood flow, or on the type of the stimulus used cannot be said yet.

The last observation in Exp. 5 was made with a direct stimulation of the gastrocnemius. The result cannot of course be compared quantitatively with those obtained when the stimulus was applied to the nerve, but the general effect is the same.

Another point of interest arising from these experiments is the comparison which can be made between the excess histamine appearing in the blood as a result of temporary occlusion of the artery and as a result of muscular contraction. Barsoum and Gaddum found that a 20-min. occlusion of the femoral artery led to an excess histamine in the venous blood of $7{\cdot}7\gamma$ for the whole limb. The weight of the limb is not given, but since the dog weighed 9·5 kg. it is safe to assume that about 700 g. would be a fair estimate for the whole limb and 500 g. for the soft tissues of the limb. This means that the average production of histamine per gram per minute was about $0{\cdot}00055\gamma$ for the whole limb and $0{\cdot}00088\gamma$ for the soft tissues. These figures must be compared with figures of the order of $0{\cdot}45\gamma$ and more as observed by us during a maximal tetanus. In other words, the effect of muscular contraction is about a thousand times greater than that of the occlusion of the artery.

The total amount of histamine set free by the whole gastrocnemius is astonishingly great. In Exp. 5 if one takes that the histamine is produced at the rate of $0{\cdot}45\gamma$ per minute per gram of muscle the total histamine produced by the muscle weighing 42 g. during its maximal contraction would be about 19γ. Barsoum and Gaddum found that during the occlusion of the femoral artery there was a production of only $0{\cdot}4\gamma$ per minute for the whole hindlimb of the dog.

Effect of "traumatized blood". Phemister and Handy [1927] have found that shaken arterial and venous blood has conspicuous vaso-dilator properties. Barsoum and Gaddum have advanced evidence to show that this is due to the production in the blood of a considerable amount of adenosine. Since, during the collection of our samples, the blood was invariably subjected to some mechanical disturbance it was necessary to show that the shaking of the blood does not affect its histamine content. For this purpose we divided our samples into two lots. One was collected with the minimal possible disturbance, while the other was subjected to a vigorous shaking for 2 min. before precipitating the proteins of the blood in the usual manner. The results were conclusive; there was no difference in the histamine content of the two lots. Active

venous blood behaved in this respect in the same way as the resting blood.

On perfusion of the muscle with blood which had been shaken for some time the histamine content of the venous blood does not increase. On the contrary, if the venous blood contained more histamine than the arterial this difference disappeared on account of the increase in the blood flow.

Do the vaso-motor nerves participate in the liberation of histamine? The tongue was chosen as the most suitable object for the study of this question. In view of the considerable collateral circulation which was always present in all our experiments on the tongue it was impossible to calculate the amount of histamine appearing in the venous blood per minute and per gram of the muscle. Nevertheless, the results of the experiments were as conclusive as those obtained with the gastrocnemius. The following experiment shows all the points of importance.

Exp. 6. Blood collected from lingual vein, the large venous anastomoses with the other side being ligatured.

Description of the blood samples	Blood flow c.c. per min.	Histamine γ per c.c.	Excess histamine γ per min.
Resting venous blood	6·6	0·018	—
Arterial blood	—	0·018	—
Rhythmical stimulation of the lingual nerve	38·0	0·018	—
Rhythmical stimulation of the vago-sympathetic nerve	3·5	0·025	0·024
Strong stimulation of the hypoglossal nerve for 5 sec.	14·5	0·050	0·464
Weak stimulation of the hypoglossal nerve for 10 sec.	7·9	0·040	0·174
Strong stimulation of the hypoglossal nerve for 2 sec.	10·0	0·030	0·120
Resting venous blood	6·3	0·018	—
Arterial blood	—	0·018	—
Venous blood during restricted circulation	3·2	0·025	0·022

Stimulation of the lingual nerve, although it produced a sixfold increase of the blood flow, had no effect on the histamine concentration of the venous blood. In those experiments in which the venous blood contained a somewhat higher concentration of histamine than the arterial blood stimulation of the lingual nerve abolished this difference. This is most probably due to the increase in the blood flow through the tongue. Stimulation of the vago-sympathetic nerve diminished the blood flow to about a half and caused an increase in the histamine concentration of the venous blood. The nerve had to be stimulated for over 3 min. before

the required amount of blood was collected, so that the excess histamine produced per minute was not great. The appearance of an excess histamine in the venous blood during the stimulation of the sympathetic nerve cannot be attributed to a specific action. It is most probably caused by the insufficient blood supply resulting from the vaso-constriction. At any rate an artificial diminution of the blood flow through the tongue caused by a partial occlusion of the lingual artery led to the same rise in histamine concentration as stimulation of the sympathetic nerve. A strong stimulation of the hypoglossal nerve for 5 sec. led to a considerable production of histamine. As in the experiments with the gastrocnemius the amount of histamine liberated per second of stimulation was larger when the muscle was stimulated for 5 sec. (0·093 γ) than for 2 sec. (0·060 γ). A weak stimulation which caused only a partial tetanus was accompanied by a much smaller liberation of histamine, although the contraction lasted for double the time as compared with the strong tetanus.

The action of curare. The action of curare upon the muscle presents some extremely complicated features which will be discussed in another communication. It is, however, important to mention here that administration of curare completely abolishes the excess production of histamine during stimulation of the muscle nerve.

Further proofs that the active substance is histamine. All the experiments described here were made with the guinea-pig ileum as a test object. The contraction of the ileum produced by the final concentrated extract of a blood sample was attributed to histamine. It would be possibly more prudent to speak rather of a histamine equivalent than of histamine proper. We would like, therefore, to emphasize that when we use the term histamine we mean an appearance in the blood of a substance which is indistinguishable from histamine in regard to its physiological effect on various test objects and in regard to its behaviour in presence of different chemical reagents. In order to verify our experiments with the help of some other test object we made use of the specific effect which histamine has on the rectal cæcum of the fowl. Some of our blood samples were estimated on the guinea-pig ileum as well as on the rectal cæcum. The quantitative estimation of such minute amounts of histamine usually gives somewhat different results as regards absolute values, but the determination of the relative concentrations of two samples should give the same result so long as these concentrations are within the range of sensitivity of the test objects. This was actually the case. For instance, a resting venous sample was estimated by the ileum method as containing

0·018γ per c.c. and by the cæcum method as 0·025γ. The active venous sample collected from the same muscle gave figures of 0·125γ with the ileum and 0·175γ with the cæcum. The important part of these tests consisted in rendering the cæcum insensitive to histamine and determining whether it also loses its sensitivity to the blood extracts. The details of the method have been described by Barsoum and Gaddum [1935*a*]. After rendering the cæcum insensitive to histamine by overdosing it with a high concentration of histamine it gave completely negative results with the blood extracts. This should be considered as extremely strong evidence in favour of the active substance appearing in the blood during muscular contraction being histamine.

In the present communication we referred to the histamine as being liberated or as being produced by the muscles during their contraction. Which of these two mechanisms actually lies at the basis of the appearance of excess histamine in the venous blood cannot be decided at present.

Conclusions.

1. No difference could be found in the histamine concentration of the arterial blood and of the venous blood emerging from a resting skeletal muscle. When the blood supply to the muscle was diminished the histamine concentration of the venous blood rose above that of the arterial blood. The excess histamine appearing in the venous blood, when the blood flow was reduced to a half of the normal, was of the order of 0·002–0·003γ per g. of muscle per minute.

2. Muscular contraction is accompanied by a considerably larger increase of the histamine concentration in the venous blood and of the total excess of histamine appearing in the blood emerging from the active muscle. The excess histamine is greater the stronger the contraction of the muscle and the longer its duration.

3. The excess histamine appearing in the venous blood, per unit of time of contraction, becomes progressively larger as the contraction continues. A maximal tetanus of a dog's gastrocnemius lasting for 10 sec. may lead to an excess histamine of about 3·5γ. When calculated per minute of contraction per gram of muscle the average excess of histamine is of the order of 0·45γ, which is several hundred times larger than the excess histamine appearing in the blood after temporary occlusion of the artery.

4. Stimulation of the vaso-dilator or of the vaso-constrictor nerves does not lead to the appearance of an excess of histamine in the venous blood.

REFERENCES.

Anrep, G. V. and von Saalfeld, E. (1935). *J. Physiol.* **85**, 375.
Barsoum, G. S. and Gaddum, J. H. (1935 *a*). *Ibid.* **85**, 1.
Barsoum, G. S. and Gaddum, J. H. (1935 *b*). *Ibid.* **85**, 13 *P.*
Gaskell, W. H. (1877). *Arb. Physiol. Inst.*, Lpz. **12**, 45.
Keller, Ch. J., Loeser, A. and Rein, H. (1930). *Z. Biol.* **90**, 260.
Krogh, A. (1922). *The Anatomy and Physiology of Capillaries.* New Haven.
Lewis, Th. (1927). *Blood Vessels of the Human Skin and their Responses.* London.
Phemister, D. B. and Handy, J. (1927). *J. Physiol.* **64**, 155.
Rigler, R. (1932). *Arch. exp. Path. Pharmak.* **167**, 54.